REMARKS

Amendments

Claims 4-8 and 12-16 are currently pending in the application.

Claims 4 and 15 have been amended to delete steps (c) through (f) solely to expedite patent prosecution in accordance with the U.S. Patent Office Business Goals (65 Fed. Reg. 54604 (September 8, 2000)). Applicants reserve the right to present the cancelled subject matter in a co-pending application.

Claims 4 and 15 have also been amended to recite "which include multipotent cells that are insensitive to neuregulin, whereby the mixed population of cells is enriched for neuronal progenitor cells." (see, *inter alia*, page 12, last paragraph; page 27, first paragraph; page 29, last paragraph; and page 32, first paragraph, page 32, last paragraph).

Claim 16 has been amended to recite "where such cells include multipotent cells that are insensitive to neuregulin" (see, *inter alia*, page 12, last paragraph; page 27, first paragraph; page 29, last paragraph; and page 32, first paragraph). These amendments are supported by the application as originally filed, and do not constitute new matter. Entry of these amendments is respectfully requested.

35 U.S.C. §112, first paragraph

Claims 4-8 and 12-16 have been rejected under 35 U.S.C. §112, first paragraph, as allegedly failing to provide adequate written description and an enabling disclosure (Office Action, page 2). The Examiner states that there is insufficient description regarding the selection of subpopulations of the RET-positive cells to allow an artisan to practice the invention as claimed (Office Action, page 4). Applicants traverse this rejection for reasons of record. However, for the purpose of expediting patent prosecution, Applicants have amended claims 4 and 15 to delete steps (c) to (f) which recite subpopulation selection (see above). Applicants reserve the right to present the cancelled subject matter in a co-pending application (see above). It is believed that this Amendment obviates the rejection under 35 U.S.C. §112, first paragraph. Withdrawal of this ground of rejection is respectfully requested.

35 U.S.C. §102(b)

The rejection of claims 8, 13-14, and 16 under 35 U.S.C. §102(b) has been maintained based on Stemple et al., 1992, *Cell* 71:973-985 ("Stemple I"; Office Action, page 3). The Examiner states that the claims are clearly anticipated by Stemple I, and that there is no factual evidence of a difference between what is disclosed by the publication and what is instantly claimed (Office Action, pages 3-4). The Examiner points to the specification on page 26, which allegedly demonstrates that RET-positive cells can be isolated by the method of Stemple I (Office Action, page 4). The Examiner also states that Stemple I teaches the production of non-neuronal cells such as glial cells, which anticipates the subject matter of claim 8. *Id.* Applicants respectfully traverse this rejection.

Applicants submit that there is clear factual evidence that the claimed cell population is distinct from the cell population of Stemple I. Simply put, Stemple I does not teach or suggest a substantially pure population of RET-positive cells. Stemple I reports a method for selecting p75-positive cells with a p75 antibody, but these cells are NCSCs (Stemple I, page 979) *which do not express RET.*

There is a large developmental gap between the rat embryos used by Stemple I and those used by Applicants. Stemple I utilizes neural crest cells from E10.5 rat embryos (embryonic day 10.5; Stemple I, page 974). In contrast, Applicants use neural crest cells from E14.5 rat embryos (page 18, lines 13-23). It is understood in the art that E10.5 and E14.5 (4 days) represent substantially different stages in rat embryo development. Those practicing in the art know that embryonic development in the rat spans only from E1 to E17.5 (17.5 days).

A notable consequence of this difference is that Stemple I E10.5 embryos give rise to RET-minus cells, while Applicants' E14.5 embryos give rise to RET-positive cells. The early cells isolated by Stemple I are identified as neural crest stem cells (NCSCs; Stemple I, page 979). The instant application states that Stemple I NCSCs are "antigenically distinct" from the cells of the invention (page 21, lines 25-26). In particular, it is noted that Stemple I NCSCs do not express RET (page 21, lines 9-20) because Stemple I NCSCs are isolated from early-stage E10.5 embryos, the cells are *too immature to express RET*, and the Stemple I methods *cannot be used to isolate RET-positive cells*. Stemple I cannot anticipate the claimed invention.

In the Office Action, it is alleged that page 26 of the instant application indicates that RET-positive cells can be isolated by the method of Stemple I. However, this is not the case. The instant application states that the Stemple I experiments were carried out with "E10.5 neural explants", while Applicants' experiments were carried out with "E14.5 neural crest-derived cells" (page 26, lines 8-13; see, also Stemple I, page 974). Again, this distinction is important. The Stemple I E10.5 embryos cannot be used to isolate RET-positive cells, because the cells *do not yet express RET.* Rather than showing the equivalence of these experiments, Applicants' disclosure makes clear the difference between the Stemple I NCSCs and Applicants' RET-positive cells. Stemple I cannot anticipate the claimed invention.

In the Office Action, it is alleged that Stemple I teaches the production of non-neuronal cells from NCSCs, and thus anticipates the subject matter of claim 8. Yet, this cannot be the case. As indicated above, Stemple I utilizes E10.5 embryos as the starting source of NCSCs. These early-stage cells do not express RET. Thus, the Stemple I cell population is clearly distinguishable from the RET-positive cell population recited in claim 16. Dependent claim 8 includes all of the limitations of independent claim 16. As such, Stemple I cannot be said to teach or suggest the subject matter of claim 8.

Applicants note that a claim is anticipated only if *each and every element* as set forth in the claim is found in a single cited reference. *Verdegaal Bros. v. Union Oil Co. of California,* 814 F.2d 628, 631 (Fed. Cir. 1987); MPEP § 2131. The cited reference must show the identical invention in *complete detail* as it is contained in the claim. *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236 (Fed. Cir. 1989); MPEP § 2131. Because Stemple I does not teach each and every element of claimed invention, it cannot anticipate independent claim 16 or dependent claims 8, 13, or 14. Withdrawal of this ground of rejection is respectfully requested.

35 U.S.C. §103(a)

The rejection of claims 4-8 and 12-16 under 35 U.S.C. §103(a) has been maintained based on Stemple I, in combination with Stemple et al., 1993, *Devel. Biol.* 159:12-23 ("Stemple II"), Lo et al., 1994, *Perspectives Devel. Neurobiol.* 2:191-200 ("Lo"), and Martucciello, 1995, *J. Pediatr. Surg.* 30:433-436 ("Martucciello"; Office Action, page 5). The Examiner states that Applicants have identified advantages that would naturally flow from the suggestions of Stemple

I, Stemple II, Lo, and Martucciello. *Id.* In particular, it is alleged that these publications teach the isolation of RET-positive cells and the study of their lineage commitment. *Id.* Applicants respectfully traverse this rejection.

To establish a *prima facie* case of obviousness, all of the claim limitations must be taught or suggested by the cited references. *In re Royka*, 490 F.2d 981 (C.C.P.A. 1974); MPEP § 2143.03. Obviousness can only be established by combining or modifying the teachings of the cited references to produce the claimed invention where there is *some teaching, suggestion, or motivation* to do so either in the references themselves or in the general knowledge of the art. *In re Kotzab*, 217 F.3d 1365, 1370 (Fed. Cir. 2000); MPEP § 2143.01.

As indicated in the previous remarks, Stemple I does not teach or suggest methods for selecting RET-positive cells. Further, Stemple I does not teach or suggest methods for selecting RET-positive cells with a RET antibody. Nor does it teach or suggest cell populations comprising RET-positive cells. Instead, Stemple I reports the isolation of NCSCs, which were known at the time of the invention to be RET-minus cells. Stemple II and Martucciello fail to remedy the defect in the Stemple I. Stemple II is a review of various environmental factors affecting growth and differentiation of neural crest development. While Stemple II make references to the use of antibodies, the use of RET antibodies was not disclosed or suggested. Like Stemple I, Stemple II does not teach or suggest methods for selecting RET-positive cells or the use of RET antibodies. Martucciello refers to immunohistochemical study of expression and localization or the RET protein in the intestinal plexuses of patients with Hirschspring's disease. Martucciello does not teach or suggest enrichment of individual cell lineages from neural crest stem cells that are RET^{+}. Martucciello only investigated tissues, not individual cells, for the expression of RET protein. Thus, Stemple I, Stemple II, and Martucciello cannot be used as primary references against the claims of the instant application.

In addition, Lo does not teach or suggest methods for selecting RET-positive cells which include *multipotent cells that are insensitive to neuregulin*. This is disclosed only by the instant application and recited in current claims 4, 15, and 16. According to the common knowledge in the art, it was expected that multipotent cells would be responsive to neuregulin, while committed cells would not (page 27, lines 15-21). Yet, the instant application demonstrates that the RET-positive progenitor cells of the invention are insensitive to neuregulin (page 27 to page

30).[1] This includes the RET-positive proneuronal progenitor cells, which are multipotent cells (page 11, lines 12-14; page 28, lines 10-13). This is a surprising and unexpected result in view of the previous findings indicating that multipotent neural crest cells were highly responsive to neuregulin (page 27, lines 12-15).

As shown by the non-responsiveness to neuregulin, Applicants have identified a unique population of cells that express the progenitor marker RET, but include both multipotent proneuronal progenitor cells (distinct from NCSCs) and committed neuronal progenitor cells (page 32, lines 2-19). Lo does not teach or suggest the selection of this specific cell population. Instead, Lo envisages a linear progression in neural crest development, showing a clear transition from uncommitted (multipotent/neuregulin-sensitive) cells to committed/differentiated (neuregulin-insensitive) cells (Lo, Figure 6). In Lo's schematic, compare the "uncommitted" top cell to the lower cells (Lo, Figure 6). Only Applicants have recognized that RET^+ uncommitted/multipotent cells and RET^+ committed cells can coexist in the same population. Applicants alone have identified multipotent cells that express RET, but are insensitive to neuregulin. This is not taught or suggested by Lo, or by Stemple I, Stemple II, or Martucciello, alone or in combination.

In this case, none of the cited references, alone or in combination, teach or suggest the claimed methods for selecting RET-positive cells which include multipotent cells that are insensitive to neuregulin. The fact that routine experiments could be carried out to identify such cells (Office Action, page 6) is not the proper inquiry. *See, Ex parte Levengood*, 28 U.S.P.Q.2d 1300; MPEP § 2143.01. Rather, there must be some motivation provided by the references or the general knowledge in the art to identify these cells. In fact, no such motivation existed. Prior to Applicants' experiments, it was expected that multipotent cells would be responsive to

[1] "As described above, some RET+ cells produced only nonneuronal cells or neurons plus nonneuronal cells, whereas others produced only neurons. This apparent heterogeneity could reflect the existence of distinct progenitor cell compartments at different and sequential stages in the lineage segregation process, as suggested for avian neural crest cells in clonal future. Alternatively, it may suggest a uniform progenitor population that exhibits clonal variation in developmental fate due to stochastic properties or to subtle variations in the local culture microenvironment. To distinguish between these possibilites, we examined the effect of recombinant human GGGFII (rhGGFII; also called neuregulin; Marchionni et al., 1993) on the behavior of these cells. . . .[T]he ratio of NP to proNPs was comparable in the two experiments suggesting that GGF [neuregulin] was unable to convert NPs to proNPs by partially inhibiting neuronal differentiation. . . The foregoing date indivated that RET+ postmigratory neural crest cells appear insensitive to GGF/neuregulin unlike NCSCs." (citations omitted).

neuregulin. Yet, Applicants discovered a surprising population of cells that includes RET-positive multipotent cells which are insensitive to neuregulin (page 32, lines 2-19). There was no teaching, suggestion, or motivation in the art to achieve this invention. Accordingly, the subject matter of claims 4-8 and 12-16 shown herein cannot be obvious in view of the cited references. Withdrawal of this rejection is requested.

CONCLUSION

Applicants believe that the claims as amended are patentable and a prompt allowance is respectfully requested. If further discussion of this case is deemed helpful, the Examiner is encouraged to contact the undersigned at the telephone number provided below, and is assured of full cooperation in progressing the instant claims to allowance.

While Applicants believe that no additional fees are required, the Commissioner is authorized to charge or credit the undersigned Deposit Account No. **50-0311**, Reference No. **18444-502**, Customer No. **35437**, for any additional fees needed.

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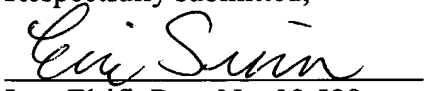
Respectfully submitted,



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